PATENT COOPERATION TREATY

REC'D 24 JUL 2001
WIPO PCT

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 4003.002310	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/US00/15243	International filing date *(day/month/year)* 02 JUNE 2000	Priority date *(day/month/year)* 04 JUNE 1999
International Patent Classification (IPC) or national classification and IPC Please See Supplemental Sheet.		
Applicant THE BOARD OF REGENTS, THE UNIVERSITY OF TEXAS		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets.

   [X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   I [X] Basis of the report

   II [ ] Priority

   III [ ] Non-establishment of report with regard to novelty, inventive step or industrial applicability

   IV [ ] Lack of unity of invention

   V [X] Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI [ ] Certain documents cited

   VII [ ] Certain defects in the international application

   VIII [ ] Certain observations on the international application

Date of submission of the demand	Date of completion of this report
04 JANUARY 2001	03 JUNE 2001
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer Dorothea Lawrence for Jane Zara
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/IPEA/409 (cover sheet) (July 1998)★

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US00/15243

I. Basis of the rep rt

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed

☒ the description:
pages 1-28 , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of

☒ the claims:
pages NONE , as originally filed
pages NONE , as amended (together with any statement) under Article 19
pages 29-31 , filed with the demand
pages NONE , filed with the letter of

☒ the drawings:
pages 1-12 , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of

☒ the sequence listing part of the description:
pages NONE , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in printed form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the writen sequence listing has been furnished.

4. ☒ The amendments have resulted in the cancellation of:

☒ the description, pages NONE

☒ the claims, Nos. NONE

☒ the drawings, sheets/~~fig~~ NONE

5. ☐ This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

*** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US00/15243

V. Reas ned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supp rting such statement

1. statement

Novelty (N)	Claims	1-22	YES
	Claims	NONE	NO
Inventive Step (IS)	Claims	1-22	YES
	Claims	NONE	NO
Industrial Applicability (IA)	Claims	1-22	YES
	Claims	NONE	NO

2. citations and explanations (Rule 70.7)

Claims 1-22 meet the criteria set out in PCT Article 33(2)-(4), because the prior art does not teach or fairly suggest compositions and methods for inhibiting estrogen-dependent tumor cell proliferation including breast cancer cell proliferation, comprising the administration of ribozymes which specifically recognize and cleave mRNA encoding a DNA binding domain of the human estrogen receptor-alpha of SEQ ID No: 4, whereby intracellular transactivation of the estrogen receptor is blocked and further whereby cell cycling of the estrogen dependent tumor cells is inhibited.

---------- NEW CITATIONS ----------

NONE

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

CLASSIFICATION:

The International Patent Classification (IPC) and/or the National classification are as listed below:

IPC(7): A01N 43/04; A61K 31/70; C12Q 1/68; C12P 19/34; C07H 21/02, 21/04, 21/00 and US Cl.: 514/44; 435/6, 91.1, 91.31, 91.5, 455, 366, 375; 536/ 23.1, 24.5, 25.3

WHAT IS CLAIMED IS:

1. A ribozyme capable of inhibiting estrogen–dependent tumor cell proliferation, said ribozyme having a high substrate specificity for an mRNA sequence encoding a DNA-binding domain of human estrogen receptor of SEQ ID NO:4, wherein said ribozyme is essentially free of endonuclease activity for an mRNA having a DNA binding domain of a glucocorticoid receptor.

2. The ribozyme of claim 1 further defined as RZ1, RZ2, RZ3, RZ4, RZ5, RZ6, RZ7, or a combination hereof.

3. The ribozyme of claim 2 further defined as RZ1 and as capable of cleaving the human estrogen receptor mRNA at a site defined further as a sequence at nucleotide position +956 herα.

4. The ribozyme of claim 1 further defined as a hammerhead ribozyme having a catalytic core with a critical sequence region, said critical sequence region defined by a sequence SEQ ID NO: 3.

5. The ribozyme of claim 2 further defined as RZ2 and as capable of cleaving the human estrogen receptor mRNA at a site defined further as a sequence at nucleotide position +894 of hERα.

6. The ribozyme of claim 1 wherein the human estrogen receptor is further defined as estrogen receptor α (ERα).

7. The ribozyme of claim 4 further defined as blocking intracellular *trans*-activation of the estrogen receptor and inhibiting cell cycling of the estrogen-dependent tumor cell.

8. A method for inhibiting estrogen-dependent tumor cell proliferation comprising:

administering a ribozyme RZ1, RZ2, RZ3, RZ4, RZ5, RZ6, RZ7, or a combination thereof to cells comprising estrogen-dependent tumor cells; and

inhibiting proliferation of estrogen-dependent tumor cells.

9. The method of claim 8 wherein the estrogen dependent tumor cell is an estrogen dependent breast cancer cell.

10. The method of claim 8 wherein the ribozyme comprises a nucleic acid sequence encoding a ribozyme RZ1, RZ2, RZ3, RZ4, RZ5,. RZ6, RZ7, or a combination thereof is administered in a vector to cells comprising estrogen-dependent tumor cells.

11. The method of claim 8 wherein the ribozyme RZ1 comprises a sequence of SEQ ID NO: 3.

12. The method of claim 8 wherein the vector is an adenovirus vector.

13. The method of claim 8 when the vector is an adeno-associated viral vector, a lentivirus, a herpes simplex virus, a liposome or a molecular conjugate.

14. A gene therapy method for reducing breast cancer cell proliferation in a cell population comprising:

preparing a pharmaceutically acceptable formulation suitable for injection systematically to an animal, wherein said formulation includes as an active ingredient a ribozyme having binding affinity for human estrogen receptor messenger RNA having a sequence as defined in SEQ ID NO:4, said ribozyme effectively reducing amounts of human estrogen receptor mRNA in said cell population;

administering said pharmaceutically acceptable formulation to said animal; and

reducing breast cancer cell proliferation.

15. The gene theapy method of claim 14 wherein ribozyme is further defined as cleaving said mRNA at a site defined at a nucleotide position of said mRNA of SEQ ID NO:4: defined at position (5):

170;	645;	1420;
190;	889;	1463;
267;	894;	1468;
377;	956;	1680;
508;	1137;	1695;
515;	1218;	1726;
543;	1240;	2077, or a combination thereof.
603;		

16. The method of claim 15 wherein said ribozyme is further defined as cleaving said mRNA at a site defined at the following position of said mRNA of SEQ ID NO:4:

377 (=RZ2)	889 (=RZ4)	894 (=RZ2)
956 (=RZ1)	1680 (=RZ5)	1695 (=RZ6)

1726 (=RZ7), or a combination thereof.

17. The method of claim 14 wherein the animal is a human.

18. A pharmaceutically acceptable formulation capable of inhibiting human breast cancer cell proliferation comprising as an active ingredient a ribozyme having

specific binding affinity to a human estrogen receptor messenger RNA sequence as defined in SEQ ID NO:4.

19. The pharmaceutically acceptable formulation of claim 18 wherein said ribozyme is further defined as specifically cleaving said human ER RNA (SEQ ID NO:4) at a site defined at position: 377; 889; 894; 956; 1240; 1680; 1695; 1726. or a combination thereof

20. A ribozyme capable of cleaving in a site specific manner a human mRNA for estrogen receptor at a site for RZ-2 at a position of said human mRNA position:

377 (RZ3); 889 (RZ4); 894 (RZ2)
956 (RZ1); 1680 (RZ5); 1695 (RZ6);
1726 (RZ7), or a combination thereof.

21. A ribozyme capable of cleaving in a site specific manner at a human estrogen sequence at position: 956,1137, 1218, 1240, 1420, 1463, 1468, 1680, 1695, 1726, 2077 of SEQ ID NO:4, or a combination thereof.

22. A ribozyme capable of cleaving in a site specific manner at a human mRNA for human estrogen receptor of a sequence at SEQ ID NO:4 at a site having a secondary structure that is positioned in an open loop region, and that is flanked on each side by an AU-rich region.